Effect of Fusarium T-2 Toxin on Hematological and Biochemical Parameters in the Rabbit

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ABSTRACT

The single intravenous administration of purified T-2 toxin to rabbits to 0.5 mg per kg body weight produced a decrease in hematocrit, white blood cell count, and serum alkaline phosphatase activity. The plasma clotting time, as measured by the activated partial thromboplastin time assay, was prolonged after intravenous T-2 toxin administration. In contrast, the administration of T-2 toxin to rabbits at 2.0 mg per kg body weight by gastric intubation produced oral lesions, diarrhea and anorexia in the animals but did not cause significant alteration in hematological and biochemical parameters. The results suggest that the rabbit may be a suitable model for further examination of the biochemical mechanisms involved in the cytotoxic action of T-2 toxin.

RÉSUMÉ

Une seule injection intraveineuse de toxine T-2 purifiée à des lapins, à raison de 0,5 mg/kg de poids vif, se traduisit par une baisse de l'hématocrite, des leucocytes et de l'activité de la phosphatase alcaline sérique. L'épreuve du temps de la thromboplastine partielle activée démontra cependant que cette dose de T-2 provoquait une prolongation du temps de coagulation du plasma. Par ailleurs, l'administration de 2 mg/kg de cette toxine à des lapins, à l'aide d'un tube oesophagien, causa des lésions buccales, de la diarrhée et de l'anorexie, sans toutefois provoquer d'altérations appréciables des paramètres hématologiques et biochimiques. Les résultats de cette expérience laissent supposer que le lapin représenterait un modèle animal adéquat pour l'étude des mécanismes biochimiques impliqués dans l'action cytotoxique de la toxine T-2.

INTRODUCTION

Fusarium trincinctum is one of the most toxic fungi isolated from mouldy corn stored under low temperature conditions. Cattle affected with corn toxicosis exhibit symptoms of epistaxis, anorexia and hemorrhagic lesions of the intestinal tract prior to death (13, 22). A hemorrhagic syndrome has also been induced in pigs fed extracts of *Fusarium trincinctum* (25) although the usual clinical conditions observed in pigs ingesting mouldy corn are associated with reproductive problems (19, 26-28). The metabolite of the *Fusarium* mould which has been implicated as being responsible for the hemorrhagic syndrome is a tricothecene derivative, T-2 toxin (4, 15-diacetoxy-8-[3 methyl-butyroloxy]-12, 13-epoxy-Δ^9-tricothecene-3-ol) (3, 14, 15).

Although T-2 toxin has been shown to induce a broad spectrum of physiological and biochemical changes in various species, the underlying mechanisms of action are poorly understood. In cats, guinea pigs and mice T-2 toxin causes decreased red cell and white cell numbers and produces altered cell morphology (8, 11, 17, 23, 24). Serum enzyme changes have been observed in chickens, pigs and a calf given T-2 toxin (5, 21, 26, 29). One difficulty in correlating results from the various studies is that not only were different species used but different dosage concentrations and different routes of administration of the T-2 toxin were employed.

Because of the potential health hazard that mycotoxins, in particular T-2 toxin, pose to farm animals it is important to understand the mechanism of action of the toxin that produces the clinical and subclinical symptoms observed in affected animals. The purpose of this study was to examine the biochemical and physiological action of T-2 toxin comparing the effectiveness of different dosages and various routes of administration of the toxin. The rabbit was selected for this investigation because of its size and ease of handling. The suitability of the rabbit as an animal model for studying the mechanism of action of T-2 toxin was evaluated since this species has not previously been used for investigation of this toxin.

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MATERIALS AND METHODS

Both male and female New Zealand white rabbits weighing between 2.0 and 2.5 kg were used. The animals were maintained under standard laboratory animal holding conditions according to guidelines established by the Canadian Council of Animal Care. Approximately five mL blood were removed from the median artery of the ear with a 23 gauge needle and collected into plastic syringes; 2.7 mL of blood was immediately transferred to a plastic tube containing 0.3 mL 0.13M sodium citrate as anticoagulant. The citrated blood was centrifuged at 2,500 x g for 20 min at 4°C to obtain platelet poor plasma which was stored at -20°C in plastic containers. The remainder of the blood was transferred to glass tubes and incubated at 37°C for two to three hours to allow clot retraction to occur. The samples were centrifuged at 2,500 x g at 21°C to obtain serum which was stored at -20°C in plastic containers.

Commercially prepared and purified T-2 toxin[1] was used throughout. For intravenous administration, the T-2 toxin was dissolved in dimethyl sulphoxide (DMSO)[2] to give a solution containing 100 mg toxin per mL. Eight rabbits were given a single infusion of this solution into the lateral ear vein at a dosage of 0.5 mg toxin per kg body weight. For oral administration, the T-2 toxin was dissolved in 1.0 mL DMSO and diluted one to 100 with sterile 0.15M NaCl to give a final concentration of 100 mg per mL. This solution was given by intubation to five rabbits at a dosage of 2.0 mg toxin per kg body weight. For each method of administration, four rabbits were used as controls and received either DMSO or the DMSO-saline solution at volumes per kg body weight equivalent to those administered to the test group. For all experiments three blood samples were taken prior to administration of the T-2 toxin. Following intravenous infusion of the toxin, samples were obtained one, two, three, four, seven, eight and nine days post-infusion at 9:00 am each day. For the oral administration of toxin, the animals were intubated on four consecutive days and blood samples taken during this period and at four day intervals thereafter up to 22 days from the start of the intubations.

Throughout the period of the experiments the weight and rectal temperature of each animal was monitored and the general physical condition rated. Daily food consumption was measured for the animals on the intubation experiments. At the completion of each experiment the animals were euthanized and examined for evidence of internal hemorrhage or gross lesions of lungs, liver, heart and kidneys. For animals included in the intubation experiments the intestinal tract was also examined. Other organs were not examined.

Hematocrit was determined on citrated whole blood samples using plain microhematocrit capillary tubes[2] and an Autocrit II centrifuge.[2] Unipettes[3] were used for white cell counts on citrated whole blood. The activated partial thromboplastin time (APTT) assay was performed on citrated platelet poor plasma using a fibrometer[3] and activated cephaloplastin[4] as previously described (9).

Serum samples were used for the determination of total protein using the Biuret reagent method (30) and serum albumin was determined by the bromcresol green method (10). For both these assay methods bovine serum albumin[5] was used to prepare standard curves using Validate and Validate-A[6] as internal quality control standards.

Serum alkaline phosphatase was determined by the method of Babson (2) using commercial reagents.[6] A standard curve was prepared using Versatol-E and Versatol-E-N[6] with Validate A[6] used as an internal control for each assay. Serum alanine amino transferase (AlAT) was determined by the method of Henry *et al* (12) using commercial reagents[2] and Validate[6] as an internal control. For each of these enzymes, the serum activity was determined on all the samples obtained from an individual rabbit at one time.

The results were statistically analysed using Student's t-test for both paired and unpaired data (1).

RESULTS

INTRAVENOUS INFUSION EXPERIMENTS

(a) *Hematological parameters* — The intravenous administration of T-2 toxin at 0.5 mg per kg body weight produced a rapid and statistically significant decrease in both hematocrit values and total white blood cell (WBC) counts (Fig. 1). By the second day post infusion the hematocrit had declined from a preinfusion value of 0.33 ± 0.02 1/1 (mean ± standard deviation) to 0.28 ± 0.01 1/1 ($p<0.05$). The hematocrit continued to decline for a further two days and thereafter began to rise toward preinfusion values. The hematocrit values of the control group treated with DMSO remained unchanged throughout the nine day period (Fig. 1). The total WBC count also declined following intravenous infusion of T-2 toxin (Fig. 1). By the third day postinfusion the WBC count had fallen to $5.4 \pm 1.3 \times 10^9$ per liter (mean ± SD) from a preinfusion value of $8.2 \pm 1.3 \times 10^9$ per liter. This drop was statistically significant, $p<0.05$. From day four the WBC began to increase towards the preinfusion value. In the control

[1]Myco-Lab Company, Chesterfield, Missouri.
[2]Fisher Scientific Co., Toronto, Ontario.
[3]Becton-Dickinson Co., Rutherford, New Jersey.
[4]Dade Diagnostics, Inc., Miami, Florida.
[5]Sigma Chemical Co., St. Louis, Missouri.
[6]General Diagnostics, Morris Plains, New Jersey.

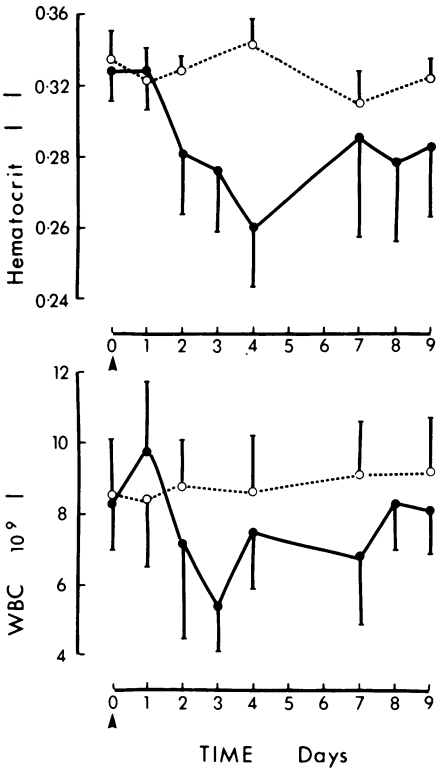

Fig. 1. Effect of a single intravenous administration (↑) of T-2 toxin (●—●) and of solvent (○---○) to rabbits on hematocrit and white blood cell counts. The results are expressed as mean ± standard deviation of seven animals.

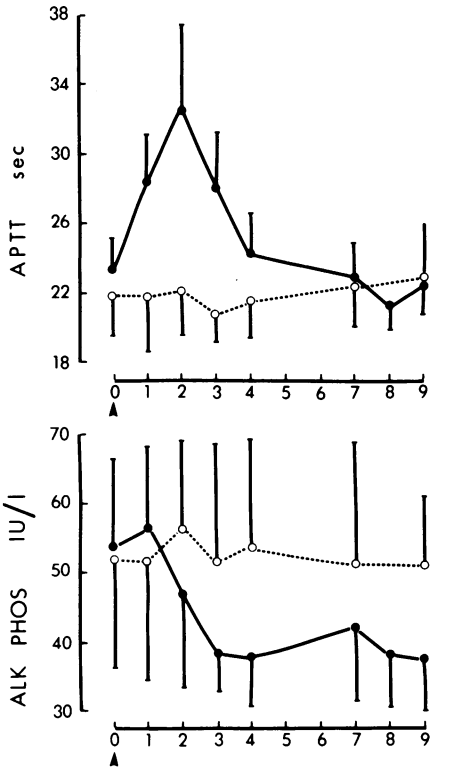

Fig. 2. Effect of a single intravenous administration (↑) of T-2 toxin (●—●) and of solvent (○---○) rabbits on activated partial thromboplastin time (APTT), and serum alkaline phosphatase (Alk. Phos.) activity. The results are expressed as mean ± standard deviation of seven animals.

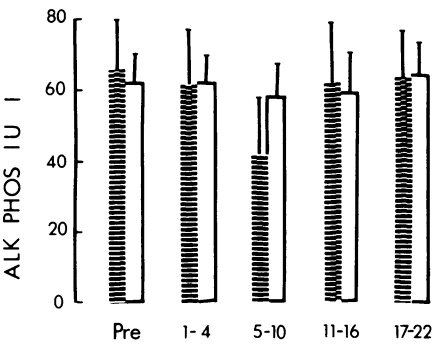

Fig. 3. Alteration in serum alkaline phosphatase (Alk. Phos.) activity in rabbits receiving T-2 toxin (enclosed bars) or solvent (open bars) by gastric intubation during days 1 to 4. The results are expressed as mean ± standard deviation. Five animals were intubated with T-2 toxin and four animals were intubated with solvent as controls.

group the WBC count remained unchanged.

(b) *Biochemical Parameters* — A prolongation of the activated partial thromboplastin time (APTT) was observed within one day of the intravenous infusion of the T-2 toxin (Fig.2). The maximum prolongation of the APTT was detected at day two postinfusion when the APTT was 32.5 ± 4.8 sec (mean ± SD) compared to an initial value of 23.4 ± 1.8 sec. The difference between these values was statistically significant ($p<0.05$). By day 4 the APTT had returned to preinfusion values. The APTT results of the control group were unchanged by DMSO treatment (Fig. 2).

A marked decline in serum alkaline phosphatase (Alk. Phos.) activity was observed in the animals treated with T-2 toxin compared to the control group (Fig.2). By the third day postinfusion the Alk. Phos. had declined from a preinfusion value of 53.8 ± 12.5 I.U. per liter (mean ± SD) to 38.3 ± 5.5 I.U. per liter. The drop in Alk. Phos. activity was statistically significant ($p<0.05$). The Alk. Phos. activity remained low in the test group for the duration of the experiment. From day 3 onward the results for the test group were significantly lower ($p<0.05$) than for the control group.

The intravenous administration of T-2 toxin produced a transient increase in serum alanine amino transferase (AlAT) activity. No significant alteration in total serum protein or serum albumin concentration was observed for either the test group of animals receiving T-2 toxin or the control group treated with DMSO.

EFFECTS OF ORAL ADMINISTRATION OF T-2 TOXIN

No changes were observed in hematocrit determinations, WBC counts or the APTT for either the test group of animals which received T-2 toxin (2 mg/kg body weight) by intubation or the control group which was given an equivalent volume of DMSO-saline solution. Similarly, no change in total serum protein or serum albumin concentration was observed for either group.

The oral administration of T-2 toxin produced a transient decline in Alk. Phos. activity five to ten days after administration of the toxin (Fig. 3). As was noted for intravenously administered toxin, orally administered T-2 toxin tended to increase serum AlAT activity. However, changes in values observed for both serum enzymes were not statistically significant compared to either initial values or values obtained for the control group.

Physical Parameters — Neither the oral nor the intravenous administration of T-2 toxin produced any change in the rectal temperature of the animals. Over the nine day period following intravenous administration of T-2 toxin the average weight of the treated animals increased from 2.30 ± 0.12 kg (mean ± SD) to 2.38 ± 0.14 kg. which was not significantly different from the control group in which the average weight increased from 2.64 ± 0.41 kg to 2.79 ± 0.27 kg. Over the three week period of the intubation experiments the weight gain in the

control group was 0.74 ± 0.05 kg (mean ± SD) which was significantly higher ($p<0.05$) than the value of 0.32 ± 0.08 kg found for the group treated with T-2 toxin. The lack of weight gain in the T-2 toxin treated animals correlates with a drop in feed consumption in the test group compared to the control group (Table I).

One of the eight animals injected intravenously with T-2 toxin was found dead 24 hours after the injection. In some animals a transient increase in respiration rate was noted 24 hours after toxin administration but otherwise no physical changes were observed. One of the five rabbits given T-2 toxin by intubation was found dead 36 hours after receiving the first dose of toxin. Autopsies were not performed on the dead animals. All the animals given T-2 toxin orally developed sores in the mucous membranes of the mouth, had hair loss, nasal discharge, and developed diarrhea within 48 hours of receiving the toxin. These problems improved once intubation ceased. No animal in the control group exhibited negative reactions to the DMSO-saline solution. When the animals were examined at postmortem no evidence of hemorrhage was observed in the T-2 toxin treated group and no gross lesions in the heart, lungs, liver, kidneys or in the intestinal tract were observed.

DISCUSSION

The results demonstrate that a single intravenous administration of T-2 toxin to rabbits at a dosage of 0.5 mg per kg body weight produces marked changes in hematocrit, total white blood cell count, plasma clotting times and alkaline phosphatase activity (Figs. 1 and 2) without inducing any apparent physical distress or discomfort to the animals. In contrast, administration of T-2 toxin by gastric intubation at a dosage of 2.0 mg per kg body weight on four consecutive days produced marked physical deterioration in the animals without significantly affecting hematological or biochemical parameters. The problems of oral lesions, diarrhea and anorexia observed in the rabbits receiving T-2 toxin by gastric intubation are identical to symptoms noted in poultry (5, 6), cats (17), guinea pigs (8) and cattle (29).

The range of hematological and biochemical parameters altered by the intravenous administration of T-2 toxin indicates that the toxin is capable of affecting several body organs. The decrease in hematocrit observed within 48 hours of the single intravenous administration of T-2 toxin to rabbits (Fig. 1) suggests that hematopoietic tissue may be affected by the toxin. It is unlikely that the drop in hematocrit was due to hemorrhage or hemolysis: no bloody discharge was observed from any orifice, the animals remained bright and alert and the plasma and serum samples showed no evidence of hemolysis during the 48 to 96 hour period when the hematocrit was reduced. In mice fed a diet containing T-2 toxin (11), hematocrit values were found to be significantly reduced seven days after the onset of ingestion of toxin. Hayes *et al* (11) elegantly demonstrated that the T-2 toxin was suppressing hematopoiesis in the bone marrow and splenic red pulp of the mice. Decreased hematocrit values (17) and cellular damage to bone marrow (23) have also been reported in cats following T-2 toxin administration. The single intravenous injection of T-2 toxin also produced a marked but transient decrease in total white blood cell count (Fig. 1). Oral administration of T-2 toxin to mice and guinea pigs also produced a decrease in circulating white cells (8, 11, 24). In mice, the lymphocyte, eosinophil and neutrophil counts all dropped in response to T-2 toxin administration and it was shown that the drop in circulating cells correlated with hypocellular lymphoid tissue (11). In guinea pigs a marked decrease in the lymphocyte content of bone was induced by T-2 toxin (8). It is probable that similar changes may have been induced by the intravenous administration of T-2 toxin to rabbits in the present study. However, since total white cell counts only were monitored and bone marrow and lymphoid tissue were not subjected to histological examination, no specific conclusions can be drawn from the results of cell count changes in the rabbit.

The most marked alteration in serum enzyme activity observed after a single intravenous injection of T-2 toxin was the decrease in alkaline phosphatase activity which occurred 48 hours after administration of the toxin (Fig. 2). Alkaline phosphatase in serum is usually of hepatic and intestinal origin. The decrease in Alk. Phos. activity could be the result of reduced synthesis of the enzyme in either or both of these organs. Since the oral administration of T-2 toxin to rabbits produces only a transient reduction in Alk. Phos. activity (Fig. 3), it is unlikely that the decrease in the enzyme activity is due to impaired intestinal synthesis of the enzyme. Studies with mice and rats have indicated that one route of elimination of T-2

TABLE I. Effect of T-2 Toxin Administered by Gastric Intubation on Feed Consumption of Rabbits

Time (days)	Average mg feed consumed per day* Control n = 4	T-2 toxin treated n = 4
Pretreatment	131.4 ± 7.3	130.7 ± 8.6
0- 4	126.5 ± 12.0	15.7 ± 17.2
5- 8	125.9 ± 5.0	89.0 ± 2.5
9-10	126.5 ± 7.7	131.8 ± 19.6
11-12	133.1 ± 2.1	132.8 ± 21.9
13-15	127.7 ± 1.2	131.3 ± 18.1
16-17	136.7 ± 1.6	133.9 ± 6.2
18-19	140.0 ± 6.8	135.7 ± 6.1

*The results are expressed as mean values ± standard deviations

toxin from the body is through the bile duct (19). Hence, the observed decrease in Alk. Phos. activity may reflect a reduction in hepatic synthesis, especially in the biliary tree, caused by the T-2 toxin or its metabolites as it is excreted from the body. A reduction in serum Alk. Phos. activity appears to be a relatively consistent response to T-2 toxin. A single intramuscular injection of T-2 toxin in chickens causes a decline in serum Alk. Phos. (21) and a decrease in this enzyme activity was one of the few biochemical changes observed in a calf intubated with T-2 toxin eleven times over a period of sixteen days (29).

One interesting finding is the dramatic prolongation in APTT results produced by intravenous T-2 toxin administration. Cattle ingesting mouldy corn infected with *Fusarium trincinctum*, the mould which produces T-2 toxin as one of its metabolites, exhibit symptoms of hemorrhagic disease (7, 13, 22, 25). T-2 toxin has been implicated as the agent responsible for the hemorrhagic syndrome (14, 16). Two recent reports have cast doubt on the significance of T-2 toxin as the hemorrhage inducing agent (18, 29). No subclinical hemorrhages could be detected in cattle intubated with purified T-2 toxin but no specific assessment of blood coagulation parameters were reported for the intubated animals (18, 29). The prolongation of the APTT found in the present study suggests that T-2 toxin is potentially able to induce a bleeding tendency. Since our data indicate that a single, intravenous dose of T-2 toxin produces a definite but transient increase in plasma clotting times (Fig. 2) of rabbits, the length of time between toxin administration and the examination of the animal may be critical for the detection of internal hemorrhages. Consequently, differences in experimental design may account for contradictory reports concerning the action of T-2 toxin as a hemorrhagic agent (18, 29).

Our data show that a single intravenous administration of T-2 toxin to rabbits produces a marked but transient change in both hematological and biochemical parameters. The transient nature of the changes may be explained, at least in part, by the fact that T-2 toxin when given to mice and rats does not appear to accumulate in any body organ and is rapidly eliminated (19). The failure to detect significant changes in either hematological or biochemical parameters following gastric intubation of rabbits with T-2 toxin is probably due to the short period over which the intubations were performed and the low blood levels of T-2 toxin achieved by the dosage given. In preliminary experiments we found that intravenous administration of T-2 toxin at dosages of up to 0.2 mg T-2 toxin per kg body weight failed to produce marked changes in any parameter in rabbits. It might also be inferred from the results that the rabbit is less susceptible to the cytotoxic effects of T-2 toxin than some other species, for example mice, guinea pigs and cats (8, 11, 17, 23).

The results indicate that the rabbit may be of use as an animal model for examining the mechanism of action of T-2 toxin since a single intravenous administration of the toxin induces some of the biochemical and hematological changes observed in other species (8, 11, 17, 21) and in cattle naturally affected with corn toxicosis (13, 22, 25).

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